The Kinetics of Biodeconcentration for Nitrate: Case Study on Microbial Denitrification and Plant Absorption

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ABSTRACT

This research was conducted to study the kinetics processes of microbial denitrification and the kinetics processes of plant absorption. The test plants were spinach, peanut and grass. The test solution contained nitrate of 24 mg N/L, glucose of 64 mg \( C_{6}H_{12}O_{6}/L \) and phosphate of 0.5 mg P/L. The results showed that the kinetics of nitrate biodeconcentration processes were exponential: \( C_{t} = C_{o} e^{-kt} \). The rate constant for microbial denitrification was stated as \( k_{d} \) which was equal to 0.0786 day\(^{-1} \). The rate constant of plant absorption was stated as \( k_{s} \), and the rates for spinach, peanut and elephant grass were 0.0745, 0.0921 and 0.1226 day\(^{-1} \), respectively. The time to process 50% of nitrate biodeconcentration efficiency (\( t_{1/2} \), as the half time of nitrate biodeconcentration, for microbial denitrification it was 8.8 days and for nitrate absorption by spinach, peanut, and elephant grass, the times were 9.2, 7.5, and 5.6 days, respectively. Therefore, elephant grass they was more effective in suppressing nitrate than peanut, denitrifier and spinach.

Keywords: Biodeconcentration, microbial denitrification, plant absorption, kinetics rate constant, half time, spinach, peanut, elephant grass

INTRODUCTION

Nitrate biodeconcentration is the process of decreasing nitrate concentration caused by living organisms, especially microbes and plants in a soil ecosystem. For sanitation systems, the nitrate biodeconcentration is processed in natural treatment systems such as evaporation and evapo-transpiration beds. Therefore, the beds serve to prevent nitrate pollution in groundwater provided that the design process, implementation and operation are well managed.

An evaporation bed is a soil layer that functions to vaporise wastewater, therefore reducing the quantity of wastewater infiltration into groundwater. As the condition of the soil layer in which microbial denitrification of nitrate occurs is saturated (Brettar and Hofle 2002; Christensen and Harremoes 1977; Ho et al. 1992), the evaporation bed can be used to suppress nitrate pollution in groundwater. An evapo-transpiration bed is similar to an evaporation bed but plants can

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be grown inside so as to cover the bed. The evapo-transpiration bed has dual functions, that is, evaporation of wastewater and absorption of wastewater by plants. Therefore, compared to evaporation bed performance, there is less wastewater infiltration and leaching of nitrate to groundwater.

Theoretically, the fate of nitrate in the evaporation bed is determined by microbial denitrification, whereas in the evapo-transpiration bed, it is determined by microbial denitrification and plant absorption. In a situation where dimension and environmental conditions for evaporation and evapo-transpiration beds are equal, what would be the kinetics of microbial denitrification and nitrate sorption by plants? The objective of this research was to investigate the kinetics of nitrate biodeconcentration for denitrifiers and different plants species and accordingly to determine the biotic species that determines the effectiveness of nitrate biodeconcentration process in the evapo-transpiration bed.

**MATERIALS AND METHODS**

*Test Solution Characteristics*

The test solution was prepared following research on the limiting factors of a sand bed reactor in the heterotrophic denitrification process (Mangkoedihardjo 2005). A test solution was made for nitrate of 150 mg NO\(_3\)\(_{-}\)/L or 24 mg N/L by dissolving 173.14 mg KNO\(_3\) into 1 L aquadest. An amount of 2.2 mg KH\(_2\)PO\(_4\) (= 0.5 mg PO\(_4\)\(^{3-}\)-P/L) and 64 mg C\(_6\)H\(_{12}\)O\(_6\)/L was added to the nitrate solution. The level of pH was maintained at 6.5 by addition of 0.1 N HCl.

An inoculant of denitrifiers was prepared by dissolving 10 g of healthy garden soil into 200 mL physiological solution made up of 8.5 g NaCl/L. One mL of the soil solution was introduced into the nitrate solution in a reaction tube containing a Durham tube. The reaction tube was incubated at 28°C for 1 week. The observation that the Durham tube contained gas at the end of the incubation period meant that denitrifiers existed and the solution was an inoculant. Ten mL of the inoculant was introduced into the test solution.

*Evaporation and Evapo-transpiration Beds*

Three evaporation beds were prepared, with each consisting of a mixture of sand 0.4 – 0.8 mm particle size and healthy garden soil. The weight proportion of sand : garden soil was 2 : 1. The mixture was placed in PVC pot ø 60 cm. A nozzle at the bottom of the pots was connected to the test solution container. The installation of the evaporation bed is shown in Fig. 1.

At the beginning of the experiment, the solution was found to flow to the evaporation bed and the level of the solution in the container was maintained at 10 cm below the surface of the evaporation bed. Addition of fresh solution was necessary to maintain an equilibrium level of solution between container and evaporation bed over the 3-day period. The condition of the evaporation bed was that of a natural soil profile, that is, with unsaturated and saturated zones.
Fig. 1. Configuration test of evaporation and evapo-transpiration beds
After equilibrium, the decreasing level of the test solution in the container was attributed to evaporation from the evaporation bed. The amount of solution loss in the container had to be measured as well as the period over which the loss occurred. Time was considered as time of process for evaporation. For example after equilibrium, if an amount of 40 L of solution was lost over 2 days, it was stated as T40 = 2 days, and if an amount of 80 L of solution was lost over 5 days, it was stated as T80 = 5 days, and so on. This experiment was carried out for T40, T80, T120, T160 and T200.

The installation and operation of the evapo-transpiration bed were similar to the evaporation bed (Fig. 1), except that in the case of the evapo-transpiration bed, plants were grown on the bed. The plants tested were spinach (Amarantus sp.), peanut (Arachis hypogaea) and elephant grass (Pennisetum purpureum). There were no specific reasons for the choice of the plant species except to study the different kinetic rates of nitrate sorption. The plants were grown in a manner such that the roots were distributed over the surface area of the bed. No fertiliser was added to make the evapo-transpiration bed comparable to the evaporation bed.

**Nitrate Content**

Soil nitrate content was measured before and after time of process. Measurements before time of process were carried out for the following: (i) the mixture of sand and healthy garden soil before equilibrium with the test solution having an intrinsic nitrate content, and (ii) the mixture of sand and healthy garden soil after equilibrium with the test solution having an amended nitrate-soil. These were similarly carried out for evaporation beds besides the test beds. Measurements after the time of process was carried out when the solution in the container was empty. This was also done for the test beds.

Soil in the bed was homogenised and 10 g of soil was taken as sample. The sample was sucked to draw the soil solution. The nitrate content of soil solution was measured by means of spectrophotometry (Dries et al. 1988; Pekdemis et al. 1998) using Spectronic-20 at λ 400 nm.

**Plant Dry Weight**

One of the reasons for the loss of nitrate from soil is nitrate sorption by plants for growth. Plant growth was ascertained by measuring plant dry weight. The dry weight was measured gravimetrically (Caicedo et al. 2000) by means of drying in an oven at 80°C for 3 days until constant weight was achieved. The dry weight measurements were carried out for plants before and after treatment.
The Kinetics of Biodeconcentration for Nitrate

TABLE 1
Process dues for evaporation and evapo-transpiration beds using different plant species

<table>
<thead>
<tr>
<th>Experimental run</th>
<th>Volume of test solution (L)</th>
<th>Be</th>
<th>Process time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bet spinach</td>
<td>Bet peanut</td>
</tr>
<tr>
<td>T40</td>
<td>40</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>T80</td>
<td>80</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>T120</td>
<td>120</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>T160</td>
<td>160</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>T200</td>
<td>200</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

Notes: Be = evaporation bed, Bet = evapo-transpiration bed, T40 = process time for 40 L of test solution to be transferred to the bed after equilibrium. T80, T120, T160 and T200 also mean the same.

TABLE 2
Nitrate biodeconcentration in evaporation and evapo-transpiration beds using different plant species

<table>
<thead>
<tr>
<th>Experimental run</th>
<th>Nitrate reduction (dC) in mg N/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Be</td>
</tr>
<tr>
<td>T40</td>
<td>5.6</td>
</tr>
<tr>
<td>T80</td>
<td>14.8</td>
</tr>
<tr>
<td>T120</td>
<td>21.7</td>
</tr>
<tr>
<td>T160</td>
<td>22.8</td>
</tr>
<tr>
<td>T200</td>
<td>24.9</td>
</tr>
</tbody>
</table>

Notes: Be = evaporation bed, Bet = evapo-transpiration bed, T40 = process time for 40 L of test solution to be transferred to the bed after equilibrium. T80, T120, T160 and T200 also mean the same.

RESULTS AND DISCUSSION

Parameters for the Kinetics
Nitrate biodeconcentration and time of process were the parameters used for determining the kinetics. Time of process was defined as a period when a fixed amount of test solution had been transferred to the beds. This calculation was used solely for evaporation or evapo-transpiration of test solution. Triplicate results of the experiment are presented in Table 1.

Table 1 shows that the time of process for evapo-transpiration beds, whatever the plant species, was shorter than the time of process for evaporation. This means that the plants contributed to absorbing the solution from the bed, resulting in a shorter time of process in the evapo-transpiration bed compared to the
evaporation bed. The different times of process found in evapo-transpiration beds might be due to the different capacity of plant species to absorb the solution.

For purposes of the study, nitrate biodeconcentration has been defined as reduction of nitrate from the beds over the specified time of process. Triplicate results of the experiment are presented in Table 2. Table 2 shows that nitrate biodeconcentration at each time of process in evapo-transpiration beds was higher than in the evaporation bed. This means that nitrate was absorbed by plants in addition to nitrate biodeconcentration by microbes. Nitrate biodeconcentration rates varied between evapo-transpiration beds perhaps due to the different capacity of plant species to absorb the nitrate. This compares with Nakamura et al. (2002) in their work with nitrogen utilisation of tropical grasses.

The Kinetics of Nitrate Biodeconcentration by Microbes
The kinetics of nitrate biodeconcentration by microbes was developed through calculation as follows: (1) \( C = C_o - dC \), where \( C \) is nitrate concentration in soil at the end of time of process. \( C_o \) is the initial nitrate concentration in soil after equilibrium which is 32.4 mg N/L (note that nitrate content of soil mixture before equilibrium with test solution was 8.4 mg N/L, after equilibrium with test solution containing 24 mg N/L, it was 32.4 mg N/L). \( dC \) is nitrate reduction in soil in the period of time of process (see Table 2, Be column). and (2) \( t \) is the time of process of T40, T80, T120, T160 and T200 (see Table 1, Be column).

The relationship between \( C \) and \( t \) is presented in Table 3. It is shown that nitrate concentration remaining at time \( t \) is nothing less than \( Ct \), 32.4 is initial nitrate concentration after equilibrium which is \( C_o \); -0.0786 is rate constant of nitrate deconcentration by microbes (kd), time \( t \) (days). Therefore, the kinetics formulae can be generalised as follows:

\[
C_t = C_0 e^{-kt \cdot t}
\]  

Eqn. 1 shows that nitrate deconcentration by microbes was kinetically exponential and followed first order reaction. This result was similar to that found by Christensen and Harremoes (1977) in their work on biological deconcentration of nitrate of sewage.

### TABLE 3
The kinetics of nitrate biodeconcentration by different biotic processes

<table>
<thead>
<tr>
<th>Biotic species</th>
<th>Kinetic equation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbes</td>
<td>( y = 32.4e^{-0.0786x} )</td>
<td>0.9465</td>
</tr>
<tr>
<td>Spinach</td>
<td>( y = 32.4e^{-0.1531x} )</td>
<td>0.9336</td>
</tr>
<tr>
<td>Peanut</td>
<td>( y = 32.4e^{-0.1707x} )</td>
<td>0.9131</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>( y = 32.4e^{-0.2012x} )</td>
<td>0.9701</td>
</tr>
</tbody>
</table>

*Note: \( y \) = nitrate concentration remaining at \( x \) (mg N/L), \( x \) = time (days)*
The Kinetics of Biodeconcentration for Nitrate

TABLE 4
The constant rates of nitrate biodeconcentration by different biotic processes

<table>
<thead>
<tr>
<th>Biotic species</th>
<th>Microbial denitrification $kd$ (day$^{-1}$)</th>
<th>Nitrate sorption by plants $k - kd = ks$ (day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbes</td>
<td>0.0786</td>
<td>$0.1531 - 0.0786 = 0.0745$</td>
</tr>
<tr>
<td>Spinach</td>
<td>-</td>
<td>$0.1707 - 0.0786 = 0.0921$</td>
</tr>
<tr>
<td>Peanut</td>
<td>-</td>
<td>$0.2012 - 0.0786 = 0.1226$</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 5
The growth kinetics of different plant species

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Kinetic equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>$y = 20.792e^{-0.0377x}$</td>
<td>0.9872</td>
</tr>
<tr>
<td>Peanut</td>
<td>$y = 25.349e^{-0.0414x}$</td>
<td>0.9794</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>$y = 15.985e^{-0.0501x}$</td>
<td>0.9876</td>
</tr>
</tbody>
</table>

Notes: $y =$ plant dry weight (g), $x =$ time (days)

The Kinetics of Nitrate Biodeconcentration by Plants

The kinetics of nitrate biodeconcentration by plants was determined based on the results of the evapo-transpiration bed for each plant species. By means of the same treatment of nitrate deconcentration by microbes, the relationships between nitrate concentration and time for each plant species is presented in Table 3. A general formula for the kinetics of nitrate deconcentration by plants is as follows:

$$C_t = C_0 e^{-kt}$$  \hspace{1cm} (2)

Eqn. 2 shows that nitrate biodeconcentration by plants was kinetically exponential and followed first order reaction, the same as nitrate deconcentration by microbes. However, the rate constant $k$ in Eqn. 2 represents a results of both microbes and plants constant rates. For the evapo-transpiration bed for spinach, peanut and elephant grass, the kinetic rates were 0.1531, 0.1707 and 0.2012 day$^{-1}$, respectively. The rate constant for plants species was determined as follows:

$$k = kd + ks$$  \hspace{1cm} (3)

where $k =$ rate constant of nitrate biodeconcentration by microbes and plants, $kd =$ rate constant of nitrate deconcentration by microbes and $ks =$ rate constant of nitrate sorption by plants species. Results of the rate constant of nitrate sorption by plant species are presented in Table 4.

Table 4 shows that the rate constant for nitrate deconcentration by microbes ($kd$) was higher than the sorption rate constant ($ks$) of spinach. However, $ks$ for peanut and elephant grass was higher than the $kd$ and $ks$ for spinach. The explanation is that nitrate biodeconcentration is controlled by biotic species.
Confirmation for the Kinetics of Nitrate Biodeconcentration by Plants

Triplicate results of plant dry weight were assessed to confirm the kinetics of nitrate biodeconcentration by plants. The results are presented in Table 5. Table 5 shows that growth kinetics of each plant species tested was exponential. The rate constant for plant growth of spinach, peanut and elephant grass were 0.0377, 0.0401 and 0.0501 day\(^{-1}\), respectively. The characteristics of plants growth were the same as nitrate sorption kinetics. This confirmed nitrate as a growth factor and that nitrate sorption kinetics is valid.

The Effectiveness of Nitrate Biodeconcentration

As mentioned previously, biotic species determine nitrate biodeconcentration. The effectiveness of nitrate biodeconcentration was further assessed by determining the time to proceed to 50 % of nitrate biodeconcentration efficiency (t\(^{1/2}\)), the so called ‘half time’ of nitrate biodeconcentration. Eqn.1 and Eqn.2 could be re-arranged as follows:

\[
\frac{C}{C_0} = 50 \% = e^{-kt/2} \]
\[
t^{1/2} = 0.693/k \]

The half time for nitrate biodeconcentration by microbes, spinach, peanut and elephant grass was 8.8, 9.2, 7.5 and 5.6 day, respectively.

CONCLUSIONS

The kinetics of nitrate biodeconcentration by microbes and plants could be concluded as follows:

1. The general formula for the kinetics of nitrate biodeconcentration by microbes and plants is \( C_i = C_o e^{kt} \).
2. Biotic species determine the kinetic rate constant (k) as well as the half time of biodeconcentration (t\(^{1/2}\)).

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